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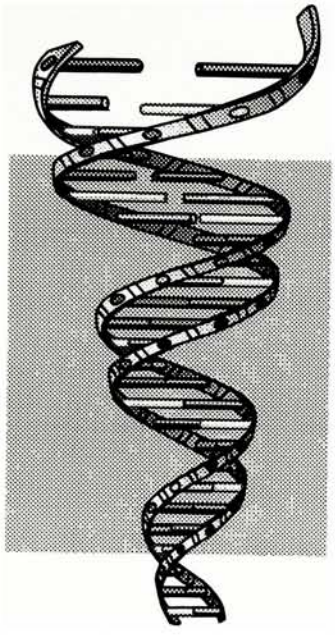
Gene therapy

Kerry Danaher



Gene Therapy

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GENE THERAPY

by

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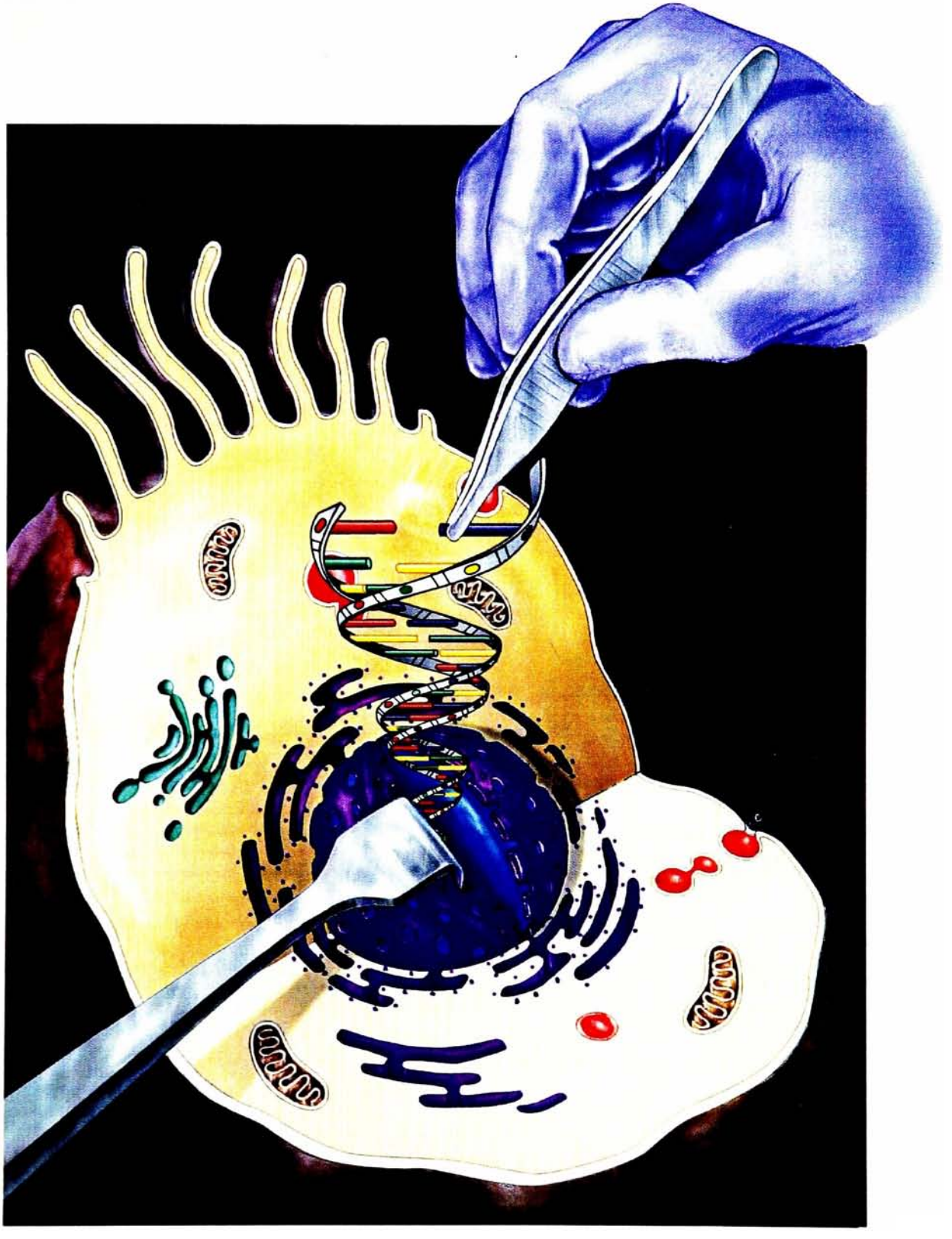

INTRODUCTION

Many advances have been made in the study of genetics over the past decade. The ultimate goal of this research is to develop medical applications which would provide a cure for the many fatal genetically linked diseases to which conventional medicine has been only able to alleviate symptoms at best.

The concept of gene therapy is based on a belief that the expression of a specific amount of functional copies of the mutated gene in the appropriate tissue could cure the disease. The Genome project is an international quest to map the entire human genome by the year 2005. This project has proven to be a major step in the science of Gene Therapy. With the mutated gene responsible for the disease located, researchers can concentrate their efforts on methods of delivering normal versions of the gene to the necessary site.

This paper looks at the developments of genetics which have led to the possibility of medical application. The four diseases illustrated in this paper are among those at the forefront of genetic research. The illustrations were created with an editorial purpose in mind. They were designed for a scientific magazine which would target a general public audience. Each disease is represented by a cover illustration, as well as a descriptive illustration of the treatment procedure which would accompany the article. The articles would be part of a four month special focus on research and developments in gene therapy.

Development of Gene Therapy

Recent clinical trials have brought Gene Therapy into the medical spot light. This modern answer to medicine actually has a fairly long history. It was born in the study of genetics, a science concerned with the inheritance of anatomical, physiological, and behavioral traits. Early founders of genetics were aware of the possibilities for future medical applications of their discoveries. Expectations and aspirations for the possible ramifications of this research has created a great interest in its development. In recent years, much time and effort has been invested in this field of research.

History

Many fundamentals of genetics developed from a growing interest in inheritance. One of the leaders in this interest was Gregor Mendel. He is considered the father of heredity. His research and studies, although viewed insignificant in his lifetime, have shed much light on the study of genetics. Much of Mendel's recognition is from experiments in plant hybridization, through his study of the pea plant. Through his research he had hoped to come to some understanding of the connection between visible characteristics and statistics of inheritance. This research offered new understandings of the basis of inheritance. The result of the studies supported Mendel's beliefs that both parents are responsible for contributions to their offspring. Prior to this time it was believed that there was a "blending" of the two sets of characteristics with the resulting offspring containing characteristics representing a means of the two. His explanation for this was that although contributions are received from both, not all are expressed. The key to this theory was the significance of dominant and recessive traits. He explained that certain units of inheritance (genes) are responsible for dictating which traits will be expressed and which ones won't.

The study of genetics is based on the physical expression of molecular makeup,

making understanding at both a micro and macro level essential to its research. Advances at the micro level began to occur opening new possibilities for the study of genetics. This new interest let to the development of cell biology. Technology supportive of this science began to develop, and in 1877 with the aid of more powerful microscopes, scientists viewed for the first time the fusion of ovum and sperm. As they studied this event they observed a thread-like structure within the nucleus of the ovum just prior to its division. This structure was what we now call the chromosome. The discovery shed a new light on genetic research. The importance of this small strand was surmised long before its function was fully understood.

In 1892 August Weismann, through his study of the relationship between parent and offspring, proposed that the genetic makeup of the offspring is the result of an equal contribution from each parent. These new findings made scientists very receptive of Mendel's work when it was rediscovered in 1900. Questions about Mendel's work and how certain genes could control the expression of others, lead to the development of a more specialized field of biochemical genetics.

In 1910 Mendel's Model of genetics was expanded upon, with the discovery that linked individual genes located on particular chromosomes to certain inherited traits. Later that year Thomas Hunt Morgan began working on an experiment similar to the one that Mendel had performed years earlier with the pea plants. Morgan took his work into the animal kingdom, studying the inheritance patterns of fruit flies. In Working with the flies he noticed that one of his males had white rather than red eyes which were normal to this species. His interest in this particular fly led to findings which suggested the existence of a recessive pattern for this characteristic linked to the male species. The characteristic visible in the male was expressed only when inherited from the female. This pattern of inheritance indicated that the gene must be located on the "x" chromosome, or is x-linked. This was the first gene that scientists were able to link to a specific chromosome. Three years later scientists had succeeded in locating the site of six more genes, thus beginning the science of gene mapping.

Further study of the chromosome led to a deeper understanding of the interchange of information obtained from a process of crossing over that occurs between a chromosome pair during sexual reproduction. This interchange of information is the result of a process called meiosis. The process of meiosis consists of two consecutive cell divisions resulting in four gametes (daughter cells) containing 23 chromosomes which is one half the number of chromosomes in any cell. When two of these gametes join in sexual reproduction each contributes its 23 chromosomes forming a new cell with a complete number of 46 chromosomes. Because of this process of reproduction the resulting offspring obtain equal contribution from each parent. This is responsible for the new combinations of hereditary traits. Studying cross over frequencies has given researchers some insight into the order and arrangement of the genes. The connection between these two factors is that closer genes are more likely to cross.

Many discoveries followed in the next few decades. In 1921 one of Morgan's students submitted a paper describing the actual makeup and function of the gene. The significance he suggested was two fold, one being its power to direct organs to produce specific substances and the second its ability to self replicate. 1940 brought further insight into the makeup of a gene with the discovery of nucleic acids as the sub units in genes. Prior to this point the existence of nucleic acids was known but they were thought to have no noteworthy function. Advances in the understanding of the function of genes and the roles they play in inheritance continued but their chemical nature was unknown until 1944. Oswald Avery made this discovery while studying an earlier theory of transforming principles in the pneumococcus bacteria. He carried out an experiment in which he selectively destroyed compounds in the bacteria thought to be possible carriers of genetic information. His observations showed that transformation persisted in all instances except with the destruction of DNA. This discovery pointed to DNA as the molecule in which genetic information is stored.

Understanding of the structure of the DNA molecule has also been essential to

genetic research. In 1953 James Watson and Francis Crick proposed a model of DNA showing its chemical structure. This model described the DNA molecule as long strands of repeating units. The strands are paired and arranged in a double helix formation. They consist of configurations of chemical sub units called nucleotides, which form rung like structures extending perpendicular from one strand to the other. There are four kinds of nucleotides repeated along the strand, distinguished by their bases: adenine, thymine, guanine, and cytosine. These bases are the variable part of the model. Variations in their sequence cause genetic differentiation. The bases are the sites where the two strands join. They have distinct shapes on their free end which function like pieces of a puzzle, thus restricting the base that can join with them. For example adenine can only join with thymine, and guanine with cytosine.

As time went on there grew a greater understanding of genetics. The basic blue print of life began to be visualized. The human body contains 100 trillion cells, these membrane bound structures are the sources of chemical reactions essential to existence. The nucleus of each of these cells contains 46 chromosomes (grouped into pairs, one from each parent) the chromosomes are filled with tightly coiled strands of DNA. DNA is made up of smaller segments called genes. The genes contain instructions to make proteins which are considered the building blocks of life. The proteins are made up of pairs of amino acids. The nucleus of each of these 100 trillion cells contains the complete DNA make up that represents the chemical description of the individual. Although each cell contains all of the genes they are not all expressed in each cell. Thus a mutation may occur in every cell but is only noteworthy in that which it is expressed.

Understanding the regulatory mechanisms of a gene is essential in genetic engineering. All cells have the same genetic makeup but the genes expressed in each varies. It is the cell regulators that are responsible for controlling which genes will be expressed. Because of this the cells can develop into different tissue and take on special functions. Proteins play an important part in this regulating system, they bind to

a gene and either block its expression or enhance it. The protein blocks expression of the regulator by processes of repression or attenuation. Repression is a block of RNA synthesis, and attenuation a halt in RNA synthesis. Expression is enhanced by the process of activation in which specific proteins are situated near promoters which help to ensure proper binding of RNA.

Information stored in DNA is transcribed to RNA where it is transformed into the essential proteins necessary for body functions. The sequence of bases on DNA is transcribed onto a messenger molecule composed of RNA. The messenger RNA carries the DNA's instructions to a ribosome, which functions as the protein factory within the cell. The new protein is then released into the cell where it can control the specific chemical reactions for which it was designed. Mutations in DNA affects the amino acid structure which in turn affects the protein. If the structure of the protein is altered enough it can become inactive. Uncorrected this mutation can be passed from generation to generation. If the mutation occurs in an essential gene it may result in the death of the organism.

GENETIC DISORDERS

Genetic mutations are permanent changes in the DNA sequence. They can either be due to single gene disorders, or chromosome disorders in which there is an excess deficiency of genes in the whole chromosome. There are many opportunities for genetic error to occur, it is not restricted to the embryonic stages of formation but poses a threat with each cycle of new cell growth. Disease results when these changes in the DNA of essential genes reduce its function below the level of that which is required. The seriousness of a disease is determined by the site of the error.

Single gene defects are the result of base change mutations. These include the exchange of one base pair for another, the insertion of one or more base pairs, or a deletion of one or more base pairs. Alterations in nucleotide sequences affect the amino acid sequence and ultimately affect production of the protein. Mutations can

occur either spontaneously, resulting from normal cellular operations or they can be induced by the interaction with different physical agents such as high-energy radiation and certain chemicals. Such agents are classified as mutagens.

Any base pair of DNA can be mutated. Point mutations are those affecting only a single base pair. They occur in one of two ways. The most common is through transition errors in which a substitution of one purine by the other or one pyrimidine by the other (G-C pair is exchanged with an A-T pair or vise versa). The other is transversion, which occurs when a purine is replaced by a pyrimidine or vise versa (A-T pair becomes a T-A pair or a C-G pair). The common cause of transitions mutagens that interact with one or more base pairs affecting their pairing properties. Another cause is base mispairing during the normal replication cycle in which the wrong base is inserted.

Another classification of mutations are insertions/deletions. Insertions of stretches of extraneous DNA via mechanisms known as transposable elements which have the ability to move from one site to another. Along with the insertion, deletions of part of the inserted material or adjacent regions often occur. One major difference between these insertion/deletions and the point mutations is that the occurrence of changes caused by transposable elements is not affected by mutagens.

One of the most common periods in which genetic error occurs is during the process of meiosis. Errors at this time often produce either numerical or structural abnormalities of the chromosome. The most common and serious type of errors are numerical abnormalities due to the fact that they directly affect the genome. Only mutations of the germline can be past to the next generation. The occurrence of these errors is usually due to nondisjunction, which is the failure of separation between a pair of chromosomes during the process of meiosis or mitosis. The result of nondisjunction is either an extra or missing chromosome. Disorders of this type are known as aneuploidy (abnormal chromosome number) which result in mental or physical mal-development. Trisomy 21 (Downs Syndrome) is the most familiar disease associated

with this disorder, it is caused by an extra copy of chromosome 13.

Other errors during the process of meiosis cause chromosome abberations resulting in structural damage to the chromosome. This damage is often in the form of chromosome breakage due to environmental factors such as radiation, drugs, chemicals, and viruses. There are several different kinds of errors that fall into this category they include:
Deletion - a section of one chromosome breaks off and its information is lost.
Duplication - a section of one chromosome breaks and attaches to the other chromosome
Inrer ion - a section of one chromosome present in reverse order.
Translocation - a transfer of one chromosome to a chromosome outside of the pair.
Crossing over - segments of a chromosome pair are exchange.

The seriousness of these errors varies. Abberations of chromosome structure, such as duplications are not as harmful because there is no genetic information lost. Those causing structural rearrangement on the other hand, such as inversions and translocations are more serious because they are likely to be transmitted to the next generation.

The result of these mutations is usually an alteration in the function and stability of the protein the gene produces. Disease is most often caused by a decrease in function or stability. It is possible for genetic mutations to enhance the function and stability however, this is less common and not indicative of a pathological effect. Proteins are created by the amino acid sequences in a gene. A deletion, addition or translation of its base pairs can disrupt production of the protein. It does so by interfering with cellular trafficking. The primary amino acid sequence contains information which allows it to be recognized by receptors located on the mitochondria membrane and imported it into the organelle. A change in this sequence makes the amino acid unrecognizable to the receptor, therefore the protein is never produced.

The severity of the disease caused by these affected proteins depends on the site and type of the protein. There are two main classes of proteins. The first is called the "house keeping" protein which is present in almost all cells and is responsible for

maintaining the structure and function of the cell. The second class consists of the specialty proteins, present in a specific number of cell types and responsible for carrying out unique tasks. Mutations in the "house keeping" proteins can express itself in one of several tissues. Mutations of the specialty proteins result in expression of disease specific to that tissue.

GENE THERAPY

New understanding of genetics has led to the manipulation of genes, altering an individual's makeup to elicit desired effects. This procedure has been in use for many years in the practice of selective breeding. Selective breeding has been common in agriculture and animal husbandry, but is now being refined to serve medical benefits. The term genetic engineering first appeared in 1932 in the paper, "Applications of genetic principles to animal and plant breeding" which was presented before the sixth International Congress of Genetics. Continuation in this direction offered hope for applications of gene therapy. Scientists began probing into the relationships between the damage of DNA and the expression of disease. Studies of the physical expression of diseases in conjunction with individual genetic make up were conducted. Through comparisons of the genetic makeup of individuals with and without expression of specific diseases, they slowly began to discover the site and type of mutation responsible for many different diseases. The term Gene Therapy refers to the application of genetic principles to the treatment of disease. With the realized connection between genetic makeup and the expression of disease it seemed logical to go directly to the source, correcting the genetic mutation rather than merely trying to treat symptoms. The basic theory behind this concept is that a functional copy of the mutated gene when expressed in the appropriate tissue could reverse expression of the disease. The futuristic ideal hoped to be obtained through genetic research is to some day be able to diagnose an illness, and treat the patient with the proper portion of genetic material to cure them.

A great deal of research and efforts have been invested in the development of such a complex proposal. Such an idea is dependent upon a greater understanding of the nature and activity of genes including their physical structure. Molecular biology has played a major role in the visualization of this physical structure. Francis Jacob and Jacques Monod began studying the expression of genes in bacteria. They discovered that a cell contains genes for many more functions then it is responsible for at a given time. Their studies indicated the existence of specialized genes in the chromosomes that function as a system of genetic regulators. These regulators are responsible for controlling each cell's function. This regulating system consists of an operator, responsible for turning on the appropriate genes, a repressor, which sits on the DNA at specific location and locks the sequence of the genes off, and operon, which controls the entire coordination sequence of the genes and the chromosome.

Gene Mapping

There are several common methods of gene manipulation essential to the practice of gene therapy. They include gene mapping, gene cloning and gene transfer. Gene mapping and cloning have been essential in the study of genetics and disease. In order for gene therapy to be considered in the treatment of a disease it is necessary that the mutated gene first be identified and its site on the chromosome located. Many advances in gene therapy are the result of this, leading to the ability to culture somatic cells in the laboratory where they can be closely studied. These studies have provided answers to the genetic links of many diseases. The identification of genetic mutations responsible for different diseases makes diagnosis and genetic council possible, and ultimately aids the development of a cure.

In efforts to advance the possibilities for the application of gene therapy scientists took on an enormous project 15 years ago of mapping the entire human genome by the anticipated year of 2005. Through this project genetic engineers are

striving to decode life's molecular secrets in order to use the knowledge to reverse the natural course of disease. The medical application of these effort is to use DNA as both a blueprint for diagnosing the disease as well as a means of correcting the mutation that make people susceptible to the disease. Their progress has been steady and many diseases have been mapped at this time. These findings have been invaluable to the recent progresses made in gene therapy.

GENE SPLICING

Research in gene therapy requires the ability to manipulate a gene, cutting some segments out and replacing them with others. The problem with this procedure had been devising an instrument small enough to function at a molecular level. The answer came in 1960 with the discovery of restricting enzymes. Research showed that foreign DNA taken in by a bacteria was broken into pieces. Bacteria uses restriction enzymes to destroy foreign DNA. This is made possible by the presence of a methyl group (CH_3) which helps the cells to distinguish between self and nonself DNA. The restriction enzymes destroy the virus DNA by cutting specific sequences of bases which it recognizes as not existing in the bacteria. Over 80 of these "restriction enzymes" have now been discovered. These enzymes provide scientists with a method of cutting specific sequences of DNA without destroying the information it carries. Another feature of this method is that when the restriction enzymes cut the DNA the free ends has an A, T, G, or C unit without a partner. This is called a "sticky" end. Segments cut with the same enzymes have complimentary sticky ends. These DNA segments look for complimentary segments to attach to. The benefit of this characteristic is that any new complimentary DNA sequence introduced after an enzyme has been cut will automatically join with the cut end.

GENE CLONING

Given the knowledge of the location of a disease's mutation scientists are able to

work toward cloning healthy versions of the gene which can be transferred into the patient to take on its designated function. The process of cloning genes was developed in 1978. This discovery has made it possible to produce large quantities of genes for research purposes which has proven essential in advancements of gene therapy. The first step to cloning a gene is isolating it. This process includes the use of specific enzymes, like the ones mentioned above, as scissors to cut the DNA into various lengths. The sequences are then separated with the use of microorganisms (micro-organisms have the ability to recognize specific sequences of DNA, they cleave to them and then digest them, thus separating them). When all the DNA has been separated the microorganism containing the segment of interest are broken and the segments removed. This procedure allows scientists to obtain sequences of interest at approximately 1 million locations.

Once isolated, it was then possible to create large quantities of genes that could be manipulated and studied. Earlier methods relied on bacteria to perform this task. The process consisted of isolating the gene, and adding it to a collection of bacteria which were stimulated to multiply. It was successful because bacteria will copy a human gene just as it copies its own. As scientists began understanding more about this formation they discovered the entire bacteria was not necessary for the job but rather specific enzymes within the bacteria called DNA polymerase. These enzymes use single stranded DNA as templates to make complimentary strands. Although this was a more efficient way to make copies, its draw back was that it could only copy single strands, meaning that the double helix had to first be separated. Exposing the DNA to heat would solve the problem of separating the helix but the amount of heat needed would also destroy the polymerase. Thus requiring a new batch of bacteria each time the process was to be repeated. The answer to this problem was to find a bacteria that was heat resistant and use its polymerase. This characteristic was found in "hot spring bacteria". The polymerase of this bacteria was accustomed to functioning at the temperature levels required for separating the helix structure. Today the process

has been refined the procedure is referred to as Polymerase Chain Reaction (PCR). In this process the genes are mixed with polymerase enzymes, heated, and then cooled until the specified number of copies are produced.

VECTORS

A common practice in gene therapy includes transferring healthy copies of the gene responsible for a mutation to the mutation site. The theory behind this is that the introduction of a healthy gene could take on production of the protein and compensate for the mutation. This method is most effective in cases which result in under expression or lack of expression of the protein. In cases where the mutation produces an over expression of the protein, the mutated gene must be inactivated before the introduction of the healthy gene. The biggest obstacle at this point in gene therapy is the development of reliable vectors for transporting the healthy gene. An efficient vector is essential because the key to this treatment lies in the ability to target a specific site. Being able to deliver the gene to the site where the genetic mutation is expressed increases the success rate, and reduces risks of random insertion activating oncogenic responses. Although there has been progress in the development of vector systems statistics show that for every gene spliced into the correct place, more than 1000 fit randomly into the genome.

Refinement of the methods for transferring gene segments has become a very important consideration in gene therapy. There are several different types of vectors used for transferring the genetic information including chemical, physical, and viral. New copies of the healthy gene are introduced into the patient via a vector by one of two ways either gene replacement or gene integration. Gene replacement, consisting of a healthy gene being placed into the spot occupied by the mutation. In gene integration a healthy gene is added anywhere in the genome. The mutated gene is not removed but the healthy gene is added to compensate for the inadequate protein production due to the mutation.

There are several different methods that can be used to transport the genes, some being more efficient than others. Chemical vectors mix DNA copies containing healthy genes with a charged substance such as calcium phosphate or certain lipids. This mixture is dumped into the faulty cell where the chemicals break through the membrane and transport the DNA inside. This is not an efficient way of integrating the gene into the genome. Usually only 1 cell in 1000 to 1000,000 will integrate. Physical vectors involve microinjection with electric shock which make the cells permeable, allowing entry of the DNA. This method is very efficient in integrating the material into the genome but the electric shock can cause damage to the cells. The method is also not as practical because only a single cell can be injected at a time. Viral vectors are the most common transporters of the genetic information at this time. They are ideal for this purpose because their natural function is to infect a cell, overtake its nucleus, and deposit its own contents into it. In the case of gene transfer the virus contents would be the healthy gene.

Viruses used in gene transfer are disabled so that they will not cause any harmful effects. The gene responsible for regulating virus replication is removed and the gene of interest along with its regulators are put in its place. The outer layer of the virus, however, containing proteins that allow it to take over other cells is left intact. The result is a structure with all of the infecting properties of a virus but no harmful viral DNA. The virus deficient of its replicating abilities must then be reproduced in a helper cell line, which provides the same function as the removed replication regulator. It is then ready to infect the target cells and insert the healthy gene.

There are several different viruses used for the transfer. They are usually chosen with consideration of the size of the gene being transferred as well as the type of gene. The most commonly used viruses are the retrovirus and adenovirus. Retroviruses are smaller and will only integrate into cells capable of actively dividing. Viral enzymes convert the RNA to DNA, helping it integrate into the genome of the host cell. Then unable to replicate the virus disappears leaving only the transferred gene

behind. Integration is not essential to the gene's function, but it offers permanent expression of the gene even after cell division has occurred. Integrating the gene into the host's genome is a more beneficial method because it provides long term expression, but it is also a riskier procedure due to the possibility of the gene integrating into the wrong part of the genome. Adenoviruses, are larger and have the advantage of being able to infect both dividing and non dividing cells, but they cannot integrate into the genome, therefore they only provide short term expression making continual treatments necessary. The adenovirus enters the host cell by binding to surface receptors. Once inside the cell it lysis the endosome and releases its own DNA. Both methods of viral transfer are used in exvivo and invivo procedures. In vitro procedures like those used in bone marrow transplants require the extraction of the cells, manipulation of their genes, and then reinjection. In vivo techniques require direct injection of the viral vector with the healthy gene into the target site.

ILLUSTRATIONS

The format I choose for my illustrations developed out of an interest in editorial illustration. I chose gene therapy as my topic because it is quickly becoming the future of medicine. It appears as a recurrent topic in all of the medical/scientific journals. As I researched the topic I found that the illustrations found in these articles are minimal and when present they are often only schematic. The illustrations were unappealing and often intimidating to the average person, full of equations with sequences of letters and numbers. With the rapid advancement in this line of research and its possibilities as an effective means of treatment it will not be long before it becomes a very big part of our medical system. It is important that the general public is made aware of these advances. As gene therapy is closer to becoming a treatment option there is a growing need to convey the procedures in a manner appealing to the general public. It was my desire through this project to design a series of illustrations that would relay the

necessary information in a simplistic manner that would be understandable to the general public, and still be interesting enough to attract their attention. I wanted to stay away from the textbook type of illustrations with molecular models and letter codes.

The illustrations would appear in a general science magazine, such as Discover or Scientific American. The cover illustrations are used as a means to draw the viewer in. The focus of these illustrations is on the patient. Using images of the patient offers the viewer something he or she can relate to and sympathize with, which helps to personalize the information making it more interesting then a collection of genetic data. The illustrations were designed to relate to each other, repeating the style and format in each helps indicate this relationship as a series.

ILLUSTRATION 1

The introductory illustration for my thesis on Gene Therapy is an 11"X14" computer generated illustration. It originated from a pencil drawing that was scanned into the computer and colorized in Photoshop 3.0. The illustration was separated into two layers, the cell and the hand. The DNA molecule was created in Illustrator 5.5 and the file was brought into Photoshop and placed on a third layer. The layer option was very helpful in this illustration because it allowed me to rearrange the placement of the images without losing the information underneath.

I wanted this piece to be more of a conceptual illustration of gene therapy, that would indicate where this research is headed. I started with a fairly textbook illustration of a cell with its nucleus and functioning components. I then altered it by adding familiar surgical elements such as a protractor drawing back the cut nucleus, and a surgeons gloved hand holding a pair of forceps. The concept behind this illustration indicates the movement of gene therapy into the practice of common medicine.

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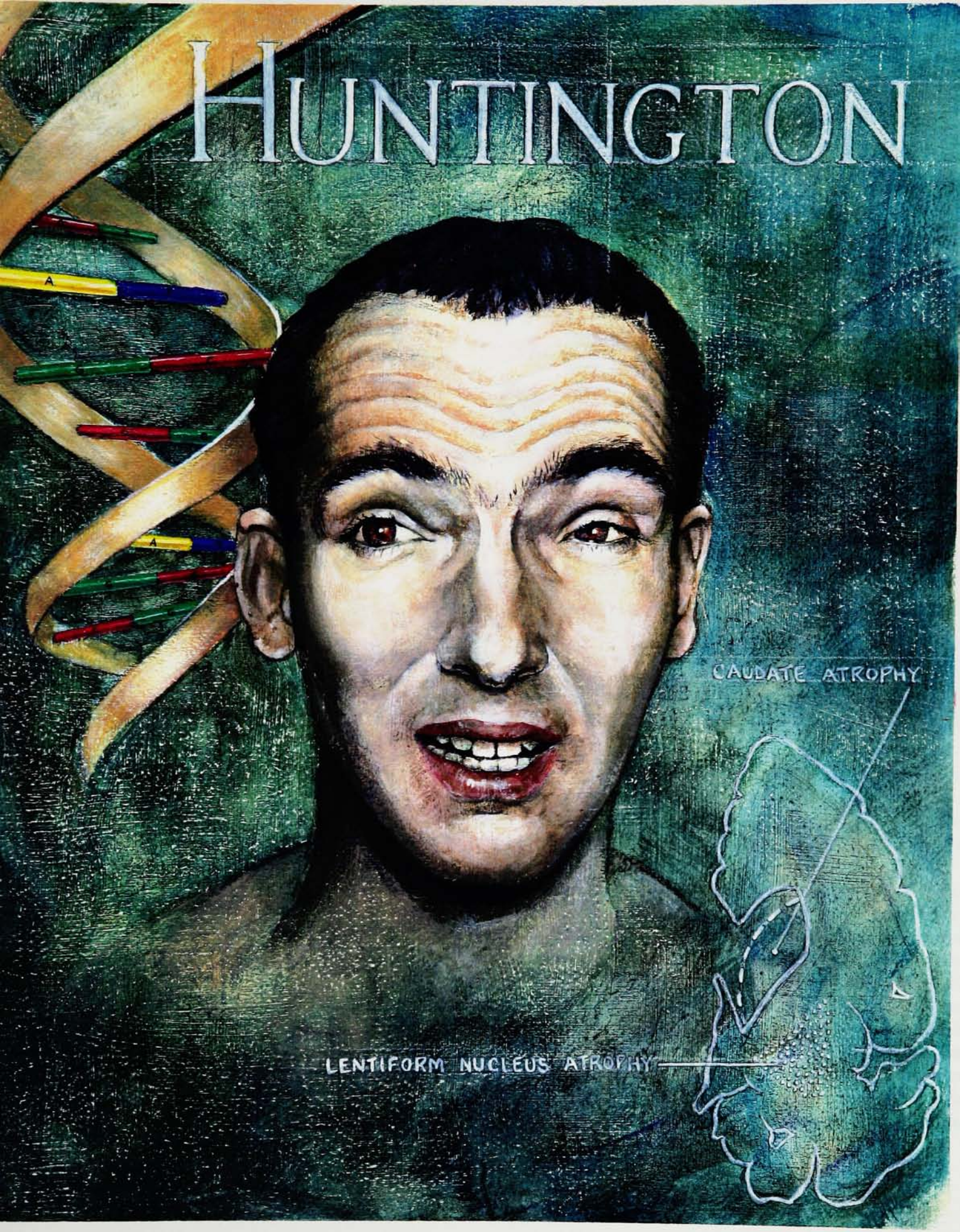
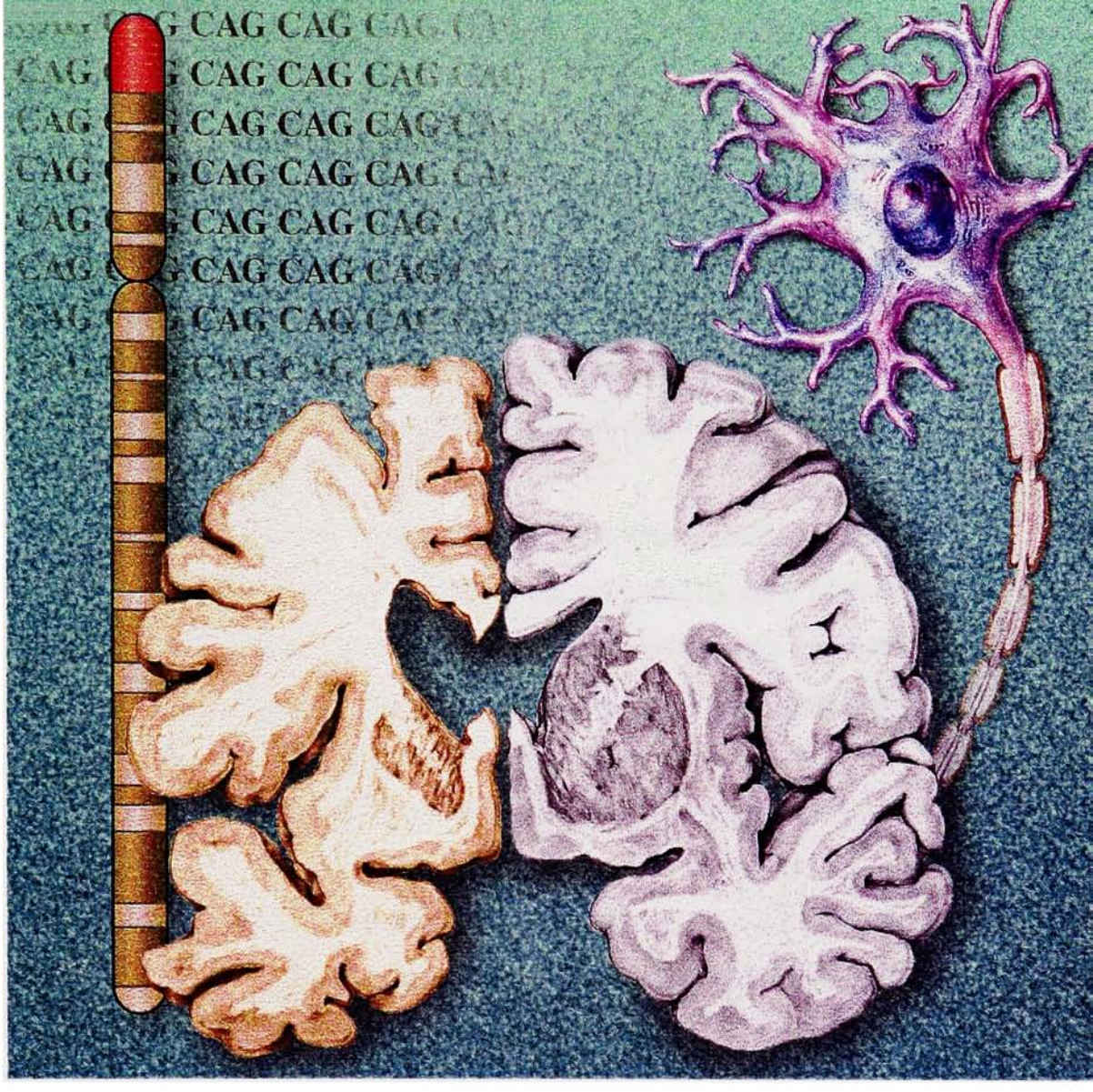

HUNTINGTON
CAUDATE ATROPHY
LENTIFORM NUCLEUS ATROPHY

Huntington's



A comparison of the brain section of a Huntington's patient on the left with a normal one on the right shows atrophy of the putamen and caudate nucleus. The atrophy is a result of neuron death linked to an increased number of CAG repeats on the tip of chromosome 4.

Huntington's disease (HD) is one of the most common and serious degenerative diseases of the central nervous system. This autosomal dominant x-linked disease expresses itself later in life with an average course of 15 years until death. The disease is a result of neuron death in the putamen and caudate nucleus, two regions of the brain which control movement. Its victims suffer from gradual muscle weakness, severe muscle spasms, chorea, body contortion, and decreased mental capacity. They eventually face complete muscle loss making even swallowing impossible.

In 1983 researchers linked HD to a faulty gene on chromosome 4. It took teams of researchers ten years before they were able to locate this gene. The gene, named IT15, was found on the tip of the short arm of chromosome 4. The mutation is an elongation of the trinucleotide repeat cytosine, adenine, and guanine (CAG). The normal IT15 gene contains a repeat sequence ranging from 10-32. Repeats above this number, usually 32-70, results in the expression of HD.

These new findings have not provided a cure at this time, but have led to more effective testing for the disease. Knowing the location and pattern of the gene allows researchers to perform presymptomatic molecular testing for diagnosis. This has led to a recent discovery of an important correlation between the number of trinucleotide repeats and the age of onset. It show patients with a higher number of repeats to have an earlier age of expression. It is now possible for doctors not only to diagnose the disease but also predict the age of onset.

HUNTINGTON'S DISEASE

Huntington's disease is one of the most common serious degenerative diseases of the central nervous system. It is an autosomal dominant disease that effects 1 in 10,000 individuals. It expresses itself later in life with no hope of treatment. There is no answer yet through gene therapy but the location of the gene responsible for the disease has made presymptomatic testing possible.

Huntington's disease (HD) is often referred to as Huntington's chorea from the Latin meaning dance. It got this name because of the violent choreography of muscle spasms associated with the disease. Huntington's is a late onset disease, expressing itself around midlife. The disease results from neuron degeneration and death in the putamen and caudate nucleus, two regions of the brain involved in controlling movement. Early symptoms include mood swings, irritability, depression, forgetfulness, and shortened attention spans. The average course of the disease is 15-20 years during which time the victim suffers from gradual muscle weakness, severe muscle spasms, chorea, body contortion and decreased muscle capacity. In the end they face complete muscle loss making even swallowing impossible.

In 1983 researchers led by Dr. Gussela located a marker for Huntington's on the short arm of chromosome 4. The presence of the marker indicated that the gene responsible for HD also resided in this vicinity. However, twenty other versions of the marker also resided there, complicating the isolation of the HD gene. Researchers began their search among the 150,000 base pairs located at the tip of chromosome 4, and continued down the chromosome over a distance of around 2 million nucleotides. Ten years after the marker was located researchers were finally abe to isolate the gene responsible for HD. Huntington's gene, technically known as "IT15", gene was located on the tip of the short arm of chromosome 4. The mutation expresses an elongation of the trinucleotide repeat cytosine, adenine, and guanine (CAG). The normal IT15 gene contains a repeat sequence ranging from 10-32. Repeats exceeding this number,

usually 32-70 result in the expression of HD.

Researchers discovered that the IT15 gene is expressed in many regions of the brain in addition to the midbrain as well as different areas of the body such as the pancreas, colon, testes, and other tissues. Degeneration, however, is restricted solely to the putamen and caudate nucleus. Not much is known of the biological affects of the disease and the significance of this repeat. Research indicates that the mRNA functions properly and is translated into normal amounts of the Huntingtin protein. The defect appears to be in the protein itself, which can be defective in one of three ways. First there can be an over activity of the protein. Second an inactivation of its normal counterpart can cause a reduction or elimination of activity in the Huntingtin protein. Third the mutation can result from an interaction of the Huntingtin protein with another cellular component. In the first two cases the Huntingtin protein is directly responsible for the disease due to a deviation from its normal function. In the third case the normal function of the Huntingtin protein is being carried out but an additional property acquired by the protein is responsible for the disease.

Research development for HD is being carried out all around the world. Researchers have been working closely and collaborating in these efforts. A study was conducted on 1007 individuals of 43 different national and ethnic groups who expressed symptoms consistent with those of HD patients. The results showed that alterations of CAG repeat did not appear to be linked to any specific nationality or ethnic group. Study of the various cases also indicated that although the disease usually expressed itself in mid life there were instances where it varied in one direction or the other. There have been victims as young as 2 and as old as 80. Comparison of variations in the age of onset with the molecular basis of the disease indicated a direct connection between the age of onset and the number of CAG repeats. It appeared that the greater the number of repeats the earlier the disease would be expressed.

These findings made it possible for doctors to perform presymptomatic tests on individuals at risk of inheriting the disease. Prior to the location of the gene in 1993, 52

indirect predictive tests and prenatal tests had been performed at the Institute of Human Genetics. The method of testing at that time relied on analysis of close DNA markers. This indirect approach required analysis of the patterns of link markers throughout the families of the individuals at risk. In 1993 with the location of the IT15 gene on chromosome 4, it became possible to directly test the individual to see if he/she had the excessive CAG repeat indicative of HD. This direct presymptomatic test for the disease offers many advantages. It is more accurate, more confidential because it doesn't require family involvement, and it is also not restrictive of individuals who do not have access to the required family data of indirect testing.

Although the genetic marker linked to HD was discovered in 1983 it wasn't until 1986 that the first presymptomatic testing began. Reservations in going ahead with the testing were due to ethical and moral concerns. Already a high level of suicide rates had been connected with HD patients. With the development of direct presymptomatic tests not only will the individual know if he has the disease but will also receive indication of when it will begin to express itself. Questions have been raised as to the benefit of being able to diagnose such a serious degenerative disease to which there is no treatment. Because of these concerns the testing is not available to everyone, and when performed is very structured. Some centers require recommendations for presymptomatic testing. Often contact with genetic councilor, neurologists, psychiatrists and psychotherapists is required and many of the larger testing centers provide long term follow ups on their patients as well. Time is also purposely allotted between the request of the test is requested and its administration because of high instances of patient withdrawal during this time. These tests are being administered in centers around the world with over 1500 people tested. This number is significantly smaller, however, than the number of individuals requesting testing. Reasons for the difference in numbers are due to either patient reconsideration during pretest counseling processes, or decline on the part of the doctors.

Although genetics offers no cure for HD to date, it has offered new a

understanding of the disease. Presymptomatic testing although risky in some aspects due to the psychological effects is beneficial for family planning because the disease is often not expressed until well into the child bearing years. In this instance testing can prevent the passing of the disease to offspring. Doctors have recognized these risks and testing conditions have been restricted to controlled environments. In the meantime research on this disease continues in hope that a method for correcting the genetic mutation lies in the near future.

ILLUSTRATION 2

The first illustration I did for this project was the cover illustration for Huntington's disease. It is an 11"X14" acrylic painting on gessoed water color paper. The paint was applied in a series of thin washes over a fixed pencil drawing. Decisions were made about which areas should be opaque and which should bleed through. In the end a fine grade sand paper was run over selective areas of the background area to set it back and add a sense of atmosphere.

I started the illustration by researching the disease and creating a list of common characteristics expressed by its victims. With this information I began developing sketches. I decided that the main focus of the illustration would be a portrait, so I began looking through pathology books for pictures of Huntington's patients. The reason for the portrait is that it draws the reader in and gives them something the can relate to. Keeping this idea in mind I chose to illustrate a patient who was not too far along with the disease. His face although showing some signs of contortion, still struggles to show the man he use to be, who 5 years earlier may have seemed no different then you or I. I demonstrated subtle contortion throughout his face to indicate expression of the disease. The eyes were an ideal site to show contortion, even slight contortions would be recognized there because it is the place that most people focus. The eye on the left side of the face is not opened as wide and the lines in the forehead

above the eye are more compressed. There is nothing relaxed about his face. The lines in the forehead indicate his strain for control. His mouth is also parched in an uncomfortable manner.

In the layout the portrait was created large to fill the foreground, so the effects of the disease would be clearly visible. My aim was to create a layout that would be visually interesting, to draw the viewer to the article. As a cover illustration it is not necessary to provide all of the information on the therapy, but attract interest and arouse enough curiosity to read the article. The DNA molecule adds an interesting graphic form, which by coming in from the corner draws the eye into the illustration. A subtle addition of letters on the rungs of the double helix suggest the CAG repeat that is indicative of the disease. The brain illustration in the lower right shows the site where the disease is expressed. Although this image is a fairly large element it does not compete with the portrait because it is a simple outline. Treating the image as an outline gives enough detail to convey the essential information without being overbearing. It is less ambiguous as to whether the image is part of the foreground or background. The decision of placement of the labels also plays a part in drawing the eye around the bottom of the illustration and backup. The hand-painted type relates to the treatment of the image of the brain as well as the title. The elements in the piece integrate well with each other. The use of a mottled background rather than a solid one makes it easier to unite the figures on it. The neck of the man and the DNA both fade into the background. Even the use of white type works well, because it is less opaque, and although readable it blends with the background. There are several elements in the painting but their treatment makes them easily recognizable as secondary to the portrait.

ILLUSTRATION 3

The computer image was created from a finished pencil drawing which was

scanned into the computer and colorized in photoshop 3.0. To the Photoshop image, an illustrator 5.5 file of the chromosome was added. The complete illustration was then brought into Quark 3.32 for layout.

The illustration was designed to be informative yet still aesthetically appealing. Staying away from solid colors I chose a speckled background, picking up colors from the cover, and fading it at its upper end. The central focus of this piece is the brain anatomy. The purpose is to offer a comparison that would show the effect of the disease in causing atrophy of the brain. I colorized the diseased section and left the healthy one black and white to demonstrate the comparison more obvious to the viewer. With no treatment yet discovered for this disease, I focused on the recent progress in presymptomatic diagnosis, now available through the discovery of the gene mutation. The illustration again focuses on conveying the mutation of the chromosome on the left with a red area indicating the site of the mutation and the CAG letters behind this area indicating the repeat sequence responsible for the disease. These two graphic elements are treated in such a way that they relate with the other more organic components of the piece. Radial transparencies were used on the type to fade them into the background yet still give enough indication of the excessive repeat. The chromosome is subdued by its color selection. The placement as well as the color of the chromosome also clearly indicates its relation to the diseased brain section and therefore its connection to Huntington's. The neuron is added because of its relationship to the atrophy of the brain. The bend in the neuron draws the eye back around to the CAG repeat. The purple color used although different from the rest of the illustration is related back as it picks up tan at the helix of the axon and then turns to grey as it fades behind the brain section. When the illustration was completed I dropped shadows behind some of the images to give a sense of spatial variation.

I played with different elements in the layout to make it more interesting. By moving the illustration to the upper right corner and letting it bleed off the page, I was able to run the title down the side of the piece in a vertical format. The vertical type

breaks up the monotony of horizontals. The choice of green type picks up colors from the illustration and the serif font adds more of an illustrative element. Vertical type is not always a good choice because it can make reading difficult, but because it was only used for the title which is a very large font size, it does not become problematic in terms of readability. The content type of the article is kept very simple using a 10 point Futura which lines up in two columns under the image. I chose a simple sanserif font because I didn't want it to compete with the actual page layout making it look too busy. For the block of type on the right side I chose a times italic to separate it from the body of type yet relate it to the title.

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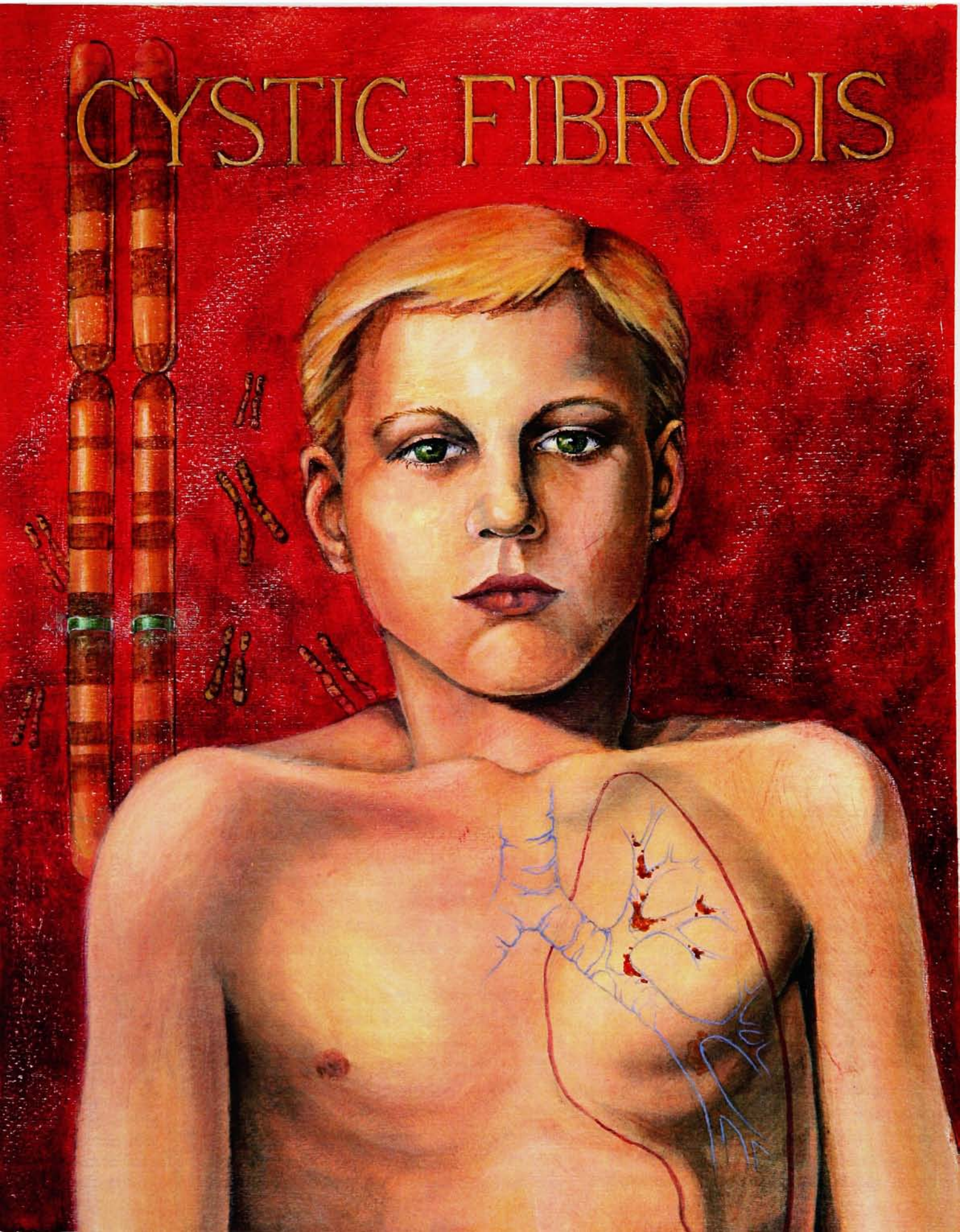
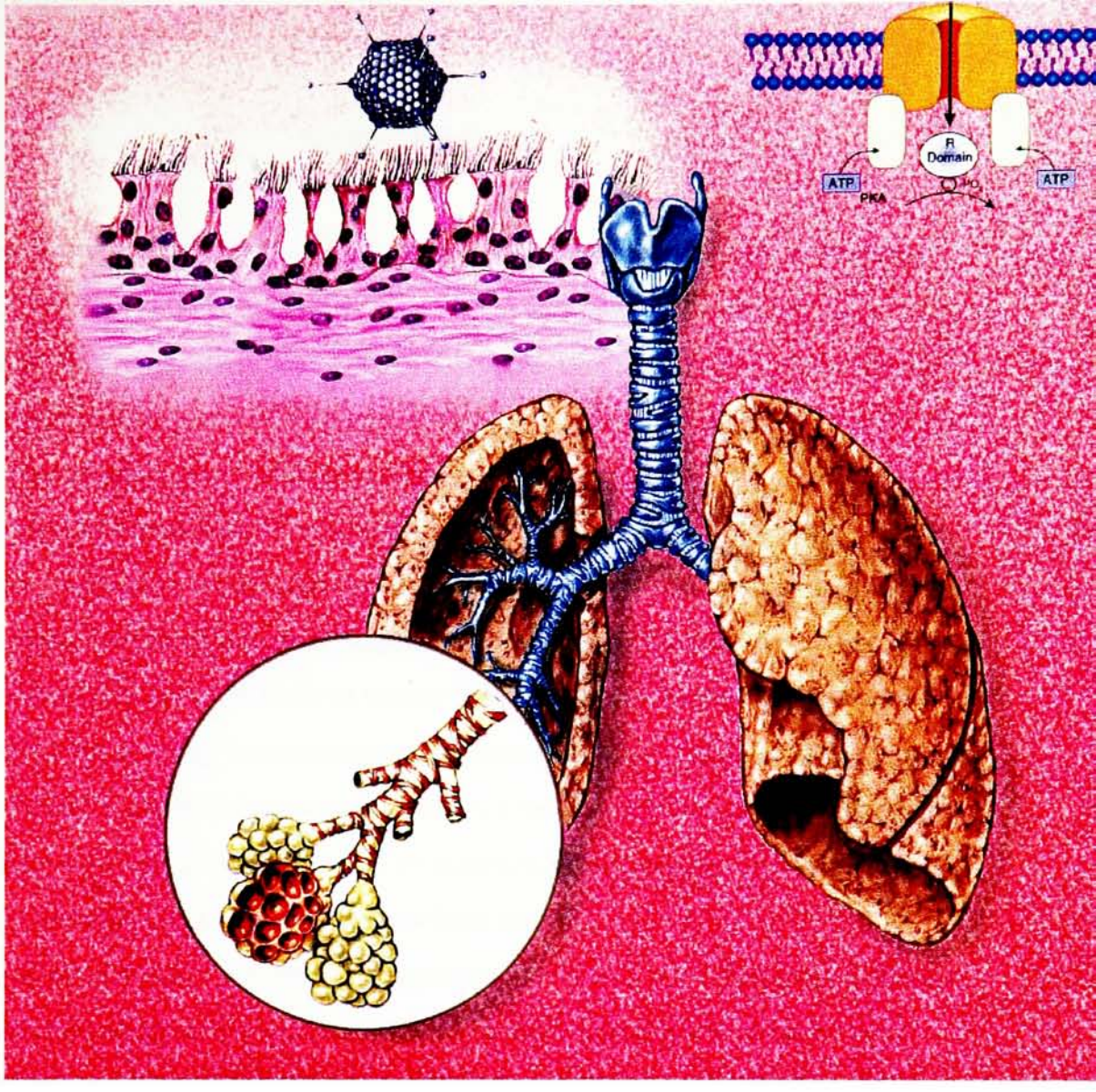
CYSTIC FIBROSIS



Cystic Fibrosis



An adenovirus carries a normal version of the CFTR gene to the lung cell epithelium. The virus binds to the cell's surface receptors and invades its nucleus. Within the cell nucleus, the CFTR protein now takes on the function of a chloride channel.

Cystic fibrosis is the most common fatal autosomal recessive disease among caucasians. The disease's primary site of expression is the lungs, where a build up of thick mucus clogs the bronchi making breathing difficult. Chronic respiratory infection and inflammation eventually lead to a loss of elasticity in lung tissue. In the end, the airsacs become unable to extract oxygen form the air.

In the 1980's, researchers linked the problem to the means by which water and salt ions, specifically chloride, pass through the epithelial cell membrane. Prevention of the normal flow of salts causes the thick mucus build - up. In 1989 the Cystic fibrosis gene was located on the middle of chromosome 7. It is responsible for making an important protein called the cystic fibrosis transmembrane conductance regulator (CFTR). CFTR acts as a chloride transfer channel, which is either absent or faulty in cystic fibrosis patients.

These discoveries opened the door for treatment with gene therapy. The plan was to transfer correct versions of the CFTR gene to the epithelial cells where they could begin functioning as chloride channels. The adenovirus, a common cold virus, became the favored vector because it naturally infects lung cells.

In 1993, Dr. Ronald Crystal preformed the CFTR transfer on a cystic fibrosis patient. Adenoviruses carrying copies of the CFTR gene were dripped down a bronchoscope into the patient's lungs. The patient's cells soon took on the production of CFTR. The trial marked a positive step but can not yet be considered an effective cure due to the short life of these cells.

Cystic Fibrosis

Cystic fibrosis is the most common fatal autosomal recessive disease among caucasians. This common hereditary disease affects around 30,000 Americans. The life expectancy for these individuals is 30 years, which is around twenty years longer then the average of ten years ago. The discovery of the gene responsible for causing the disease has opened new doors for treatment with gene therapy.

The disease's primary site of expression is the lungs, where a buildup of thick mucous clogs the bronchus making breathing difficult. This constant mucous build up provides an ideal breeding ground for bacteria and infection. Treatment for this has been pounding on the patient's back to loosen the mucous and dislodge it from the airways. Antibiotics are also administered to reduce risks of infection. Despite these efforts patients still suffer from chronic respiratory infection. Inflammation caused by these infections eventually leads to a loss of elasticity in lung tissue. In the end the air sacs become incapable of extracting oxygen from the air.

Several other organs are also affected by the disease. As in the lungs a similar thick fluid builds up at several sites throughout the body blocking small tubes and causing disfunction of their related organs. In the small tubes of the pancreas build up of this fluid blocks the flow of digestive enzymes, making patients dependent upon continual treatment with enzyme supplements to enable them to digest their food. The fluid building up in the liver clogs the ducts, and therefore poses a threat of organ failure. In males the vas deferens is a common site for fluid buildup which often leads to infertility.

The link between the different organs affected is that they all rely on epithelial tissue. Based on this, researchers searched for a defect linked to the epithelium. The production of salty sweat as a recurrent characteristic in cystic fibrosis patients led researchers to take a closer look at the process of chloride ion transport across the membrane of epithelial cells. In experiments conducted in the 1980's researcher's

linked the problem to the means by which water and salt ions, specifically chloride pass through the epithelial cell membrane. Cystic Fibrosis results when the cystic fibrosis transport regulator (CFTR) protein is damaged or absent. This defect results in an unbalanced exchange of ions, with the number of ions leaving the cell being too low. The ion transfer is directly related to water transfer. Water travels to the site of ion accumulation to dilute it. In a functioning system greater concentrations of ions are present outside the cell which draws the water out. With a faulty system in which ions build up within the cell the water stays inside. Insufficient amounts of water transferred through the cell into the lumen of the related vessels and organs results in an inability to dilute the fluids produced by the organs. The result of this is a thick mucous build up and which clogs small passage ways.

Progress began to quicken with greater insight and understandings of the disease. In 1985 Francis Collins from the University of Michigan and Lap Chee Tsui from the University of Toronto began using markers to locate the CF gene. They isolated the gene between two markers that were located 1.6 million base pairs apart. In 1989 they located the gene on the middle of chromosome seven. This gene is responsible for making the important CFTR protein which is absent or defective in cystic fibrosis patients. With the gene responsible for the disease located applications of gene therapy were now a possibility.

Plans for gene therapy began to develop based on a belief that correcting the gene could cure the disease. One factor that makes cystic fibrosis such a desirable candidate for gene therapy is that very little of the CF protein is necessary to restore proper ion transport. This is beneficial to scientists because current methods of transferring genes are not consistent in directly targeting specific cells.

Inspired by Andrea Pavivani's research of the adenovirus as a vector for transferring genes, Ron Crystal of the National Heart, Lung and Blood Institute in Washington DC. began to realize its possibilities in cystic fibrosis. The adenovirus better known as the cold virus naturally infects lung cells. This invivo approach to gene

transfer, in which the normal gene is transferred directly into the patient, eliminates the need for cell extraction and lab culture. Crystal and his colleagues began working on this concept and developed an adenovirus suitable for the transfer. The virus was disabled so it would not replicate, and a healthy gene was inserted. On April 17, 1993 Crystal brought this procedure to human trial. Modified adenoviruses carrying normal copies of the CFTR gene were dripped down a bronchioscope into the patients lungs. As expected the adenovirus infected lung cells and transported healthy copies of the CFTR gene to its nucleus where it could theoretically correct the problem.

Crystal has treated several other patients with this technique. He has had success with all of his patients with the exception of one case which was quickly rectified. In this case a woman began to express toxic side effects due to too high of a dose of the virus. There is still some experimentation that needs to be done to find the balance between too much and too little of the virus. Slight variations of this technique are being carried out by other doctors. One procedure being performed by Welsh at the University of North Carolina transfers the virus by dripping it into the patients nose rather than their lungs. Nasal epithelium appears to produce the same results. Function of the ion pump is restored. The benefit of targeting nasal epithelium is that it is more accessible.

There is still much work that must go into developing a more efficient method of transferring the gene. Although this adenovirus has been successful in restoring function of the ion pump, it does not integrate into the cell's genome. Because it is not integrated, after around three months when the epithelial cells are shed the patient faces the need to repeat the treatment.

ILLUSTRATIONS 4

The cover illustration for Cystic Fibrosis is an 11"X14" acrylic painting on gessoed paper. It was created in the same style as the previous cover illustrations, by

thin washes of paint built up over a fixed pencil drawing. I used a rich red for the background and treated the boy with very warm colors as well. Some of the golden colors from the DMD piece were also incorporated into this painting.

The portrait for this piece is a child because as I researched the fact that disease and looked through pathology books most of the patients were children due to the disease has an early age of onset and shortened life expectancy. I widen the view of this portrait to include the chest because it is one of the major sites where the disease is expressed. Outward distortions are seen in the area of the upper chest, exhibiting what is known as a barrel chest which is a common characteristic among these patients. Including the chest in my illustration also allowed me to show the inner expression of the disease. I added a simple schematic illustration over the left side of his chest to show what occurs in patients with the disease. The lung was also included to help orient the viewer. The brown accumulations in the bronchus indicates mucous build up.

In the background I added two chromosomes running vertically down the left side of the paper. Placement of the chromosomes on the left helps balance the schematic illustration in the right lower corner. Glowing green sections on the chromosomes indicate the sight of the mutation. Repetition of the more organic microscopic views of chromosomes behind the larger graphic one were added as a design element to fill the space. In deciding on a color for the type I was restricted to lighter shades because of the dark background. The white that I used in two of my other pieces would have been too harsh for this one, so I chose to pick up the gold. I finished the piece by lightly sanding the background to set everything back slightly.

ILLUSTRATION 5

The computer piece was created in a similar way as the other three, by scanning a pencil illustrations into photoshop 3.0. The schematic illustration of a chloride

channel was created in Illustrator 5.5 and imported into Photoshop. In photoshop I arranged the images on different layers and when the illustration was completed I brought the file into Quark 3.32 to create the layout.

Using the same type of background as the other computer illustrations I changed the color to pick up the reds in the painting. The main focus of this illustration is the bronchus and lung structure because it is the site that the treatment targets. I enlarged the image of the lungs and bronchi and placed them fairly central on the page to indicate their importance. The cut section of the right lung allows the viewer to follow the bronchi as they branch into smaller segments. I continued the branching beyond what is visible to the eye by adding an inset of the bronchioles with termination at the aveoli. This shows the site where mucous easily builds and causes obstruction due to its small size.

The histology plate at the top left corner shows the procedure in which the new gene is transferred in an adenovirus to the epithelial cells. I faded the edges of the image into the background to drop it back and reduce competition with the lungs. Because of the area that this image needed on the left side of the page I shifted the lungs from a central position to the right slightly. This shift also helped to fill the space on the right side of the page and while giving a little more space to the side of the aveoli. The Illustrator file in the upper right corner shows the faulty function of the mutated gene which is corrected by the transfer. This element works well visually because it fills the space in the corner without over powering the area. Treating it as a simple schematic helped to set it back and also allowed me to reduce the size a good amount without loosing information. I dropped a shadow behind the lungs and inset to bring them to the foreground and set it apart slightly.

The layout for the piece is similar to the others, the illustration bleeds off the top right corner, two columns of 10 point futura type lines up under the illustration, and a small block of 10 point Times italic on the left side describes the illustration. The title of the article runs vertical up the side and picks up the color of the illustration.

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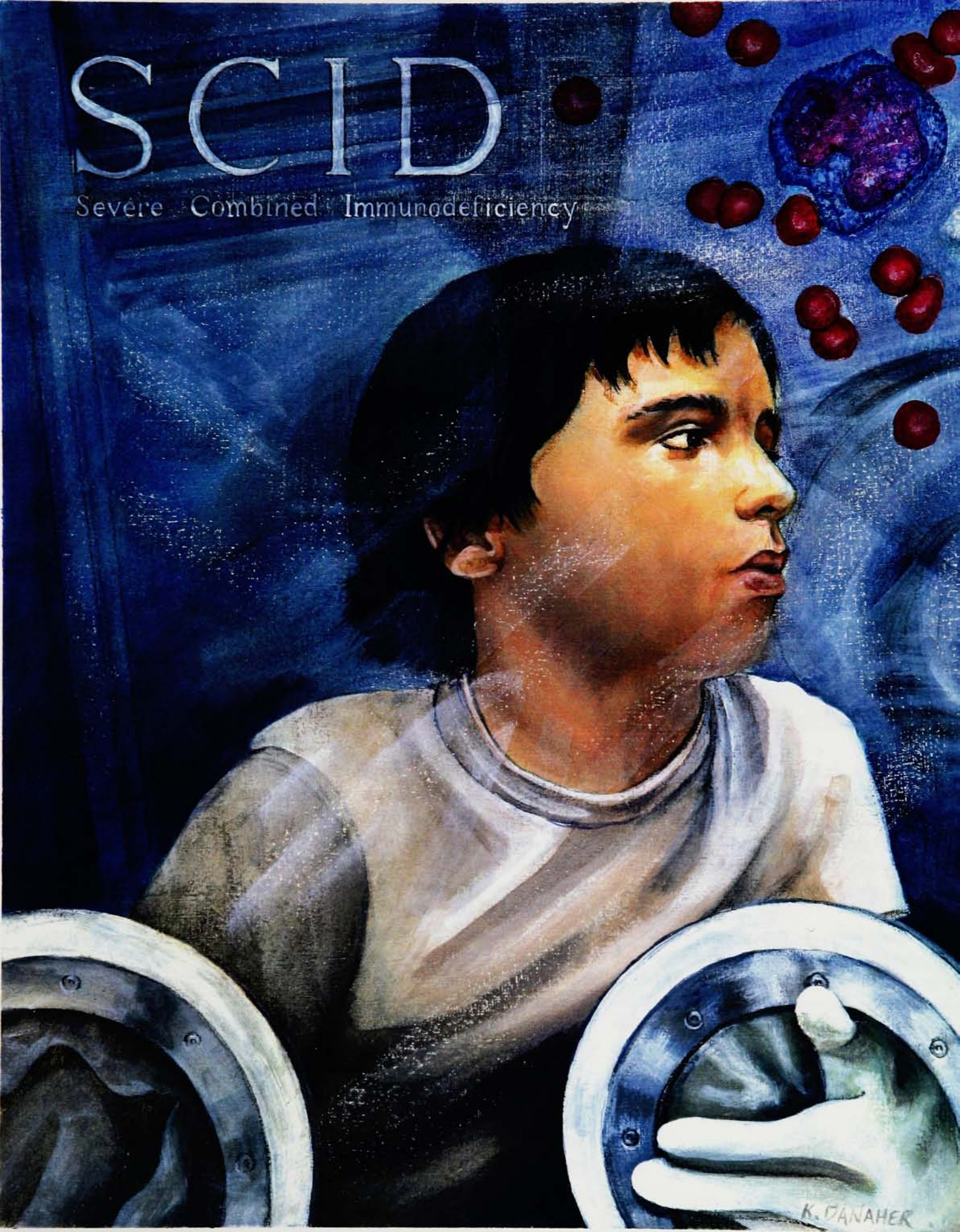
SCID
Severe Combined Immunodeficiency
K. DANAHER

S.C.I.D.

Severe Combined Immunodeficiency

Blood is drawn from the infant's umbilical cord, and the stem-cells are isolated. A retro-virus is used to carry a normal copy of the ADA gene to the stem-cell's nucleus. The stem-cells are re-injected into the baby's blood stream, where they migrate to the bone marrow and take on the task of blood cell production.

Severe Combined immunodeficiency (SCID) is an autosomal recessive disorder, caused by a blocked production of adenosine deaminase (ADA), an essential enzyme which breaks down harmful bodily chemicals. The reduction of ADA leads to a build up of these chemicals, which poison the immune system's T-cells leaving the body severely susceptible to disease.

SCID received much recognition in the 1970's through the tragic story of David, the famous "bubble boy", who spent the twelve short years of his life sealed in a plastic enclosure. Many advances have been made in the study of genetically inherited diseases, and treatment has come along way from the plastic bubble. SCID was the first disease ever treated with human-gene therapy. In a 1990 trial, T-cells extracted from a patient were given normal versions of the ADA gene via retrovirus vectors and then reinjected. The success of this procedure was the T-cell's ability to take on the production of ADA, enabling the immune system to function properly. Although an effective treatment, the short life of the T-cell would make it necessary to repeat the treatment every few months.

Searching for a cure, researchers turned their focus to the stem cells, long-lived precursors of all blood cells. Correcting the mutation at this site would lead to a continual production of healthy T-cells. Stem cells, although difficult to isolate in adults are plentiful in umbilical cord blood, making this procedure ideal in cases of prenatal diagnosis.

Severe Combined Immunodeficiency

S C I D

Severe Combined Immunodeficiency (SCID) is a rare autosomal recessive disorder affecting less then 1 in 100,000 people. A common form of this is adenosine deaminase (ADA) deficiency. The function of ADA is to protect the body from toxic chemicals that can harm the body's T and B-cells. The result of ADA deficiency is an extremely low functioning immune system that leaves its victims severely susceptible disease. New hope for this fatal disease is now under trial with the help of gene therapy.

SCID received much recognition in the 1970's through the tragic story of David the famous "bubble boy". The disease expresses itself through constant bouts with infection. Most patients are diagnosed with the disease by age two, due to continual trips to the hospital. Children undiagnosed and untreated usually die by the age of one or two from infection. The only means of treatment had been to create a way to keep the patient out of contact with germs. This was attempted by sealing them in a sterile environment. Children like David were often placed inside a small plastic enclosure. Even the extremity of this method was of little match against the disease. David died after spending twelve years inside the bubble. Most patients are considered lucky if they make it to their teenage years. .

The ADA gene is expressed mainly in the thymus and other lymphoid tissue. ADA is a protective enzyme that restricts the build up of other biochemicals that can have toxic effects on the body. In the absence of ADA, adenosine, deoxyadenosine (dADO), and deoxy-ATP builds up in excessive levels in body tissue and fluids. The excess of dADO inhibits DNA synthesis which leads to cell death. The main site of cell death is the immune system's T-cell, however B-cells are also affected. The body relies on the T-cells for protection against antigens. A deficiency or absence of this defense system leaves the patients severely susceptible to disease making even the common

cold a life threatening condition.

Science has come a long way since the boy in the bubble. Treatment for the disease has included bone marrow transplants, enzyme replacement, drugs, and now gene therapy. Without knowing much about the disease and its causes the only treatment doctors could offer was isolation, restricting the patient to a sterile environment to keep the germs away. As researchers began looking for ways to treat the disease bone marrow became an important focus because it is the site of the ADA production prior to its entrance into the blood stream. Ideas of bone marrow transplant were suggested but the problem with this treatment is the inaccessibility of compatible bone marrow. Less then 30% of the patients have a tissue compatible donor. Enzyme replacement also had its drawbacks in that it can't produce total immune system reconstruction. Drugs have also been used to stimulate the immune system and alleviate some of the effects of the disease.

Recently researchers at a biochemical company in New Jersey developed Adagen, a drug that would simulate the function of ADA. The drug was created by altering ADA from cows and making it acceptable to humans. Weekly injections of Adagen were given to a girl suffering from chronic infections due to ADA. The girl's infections ceased indicating the possibility of a new successful treatment. Upon continual use of Adagen however, the drug began to express an inconsistency that raised questions of its effectiveness. In some instances although their immune system was enhanced other areas were affected. One child lost the ability to clot normally, some children got worse and two actually died as a result of the drug. In the last case the drug stimulated the child's immune system to the degree that it attacked its own red blood cells. These treatments aimed at alleviating the symptoms and bringing the patients out of the bubble, were by no means a cure. They required endless trips to the hospital for repeated treatment with no assurance that each treatment would be successful. Although the treatments were limited in their effectiveness, the production of some amount of the missing enzyme is better then none and so treatment continued

as researchers continued their search for a cure.

New discoveries in the area of genetics has offered new hope for those with ADA. The first of these was the location of the ADA gene. This was found at the tip of the short arm of chromosome 20. The discovery made it possible for researchers to clone and study the defect. Researchers held to the belief that if they could replace this defective gene with a normal version they could cure the disease. Continuation in this line of research was supported by the refinement of vector systems.

In 1983 efforts went full force. W. French Andersen at the National Institutes of Health (NIH) concluded from his research that ADA was the best disease for testing gene transfer techniques. Possibilities of using virus vectors were supported by Richard Mulligan's 1981 experiment involving the successful transfer of human genes into mouse DNA by mouse leukemia retroviruses. This technique was refined and in 1989 the method was tried in a laboratory on samples of blood from two Ohio girls with ADA deficiency. Micheal Blaese and Kenneth Culver performed the experiment, using retroviruses. The viruses carrying normal versions of ADA were transferred to the girl's defective blood cells. The result of this landmark experiment was the production of ADA by the implanted gene.

The success of this experiment led to the first approved human trial of gene therapy. On September 14, 1990 one of the two Ohio girls, Ashanti Desilva age four was the first person ever to be treated with gene therapy. Blaese, Anderson and Culver worked together on this case. They removed the girl's white blood cells and using a retroviral vector transferred the healthy gene into the cells. The altered cells were then placed in a plastic bag and were slowly transferred back into Ashanti through an intravenous tube. The results was positive and on January 30, 1991 the same treatment was performed on the second Ohio girl, Cynthia Catshalt age four. Cynthia also showed success. In both cases almost all symptoms of the disease were relieved and the girls were now even able to leave their houses. The effectiveness of this treatment will last as long as the new cells circulate in the body. The drawback with

targeting the white blood cells is that they have a short life span, usually only several months. Because this is a genetic disease, as the altered cells die the body will produce new ones using its original genetic plan, thus the patient once again is faced with an ADA deficiency. Although this trial didn't provide a long term cure it shed new light on the possibilities of gene therapy. Being able to transfer a gene and having it take on its programmed function provided hope not only for this disease but other inherited diseases as well.

Excited by their progress Anderson, Blaese and Culver joined with two blood experts from NIH, Arthur Nienhuis and Cynthia Dunbar. Their new efforts were based on the belief that if the gene could be corrected in the stem cells (the precursors of blood cells) it could produce healthy white blood cells. The advantage of targeting the stem cells, is that unlike the white blood cells they remain in the body for life. Therefore correction at this site could permanently rid the patient of the disease. The difficulty of this method is that the stem cells are not abundant and are difficult to isolate. In May of 1993 Cynthia, one of the Chicago girls was treated by this new method that targeted the stem cells. Cynthia was given a drug that drew her stem cells out of the bone marrow into the blood where they could be drawn. The stem cells were then isolated and normal copies of the gene were transferred to the cells by a retrovirus. The altered cells were reinjected into the blood stream in hope that they would migrate back to the bone marrow. The procedure appeared to be a success but their methods of obtaining the cells still needed some refinement.

Their next efforts were based on the fact that there is one place that the stem cells are plentiful and that is in umbilical cord blood. A new procedure utilizing the umbilical blood was quickly developed and brought to trial. Two newborns who had been diagnosed with having the disease through amniocentesis were ideal candidates. The first patient was Andrew Bobea. The moment he was born his umbilical cord was snipped and blood drawn from it. The blood was sent to the Children's Hospital in Los Angeles, where Donald Hohn and Kenneth Weinberg isolated the stem cells and

transferred the new genes. The cells were reinjected back into the infants blood stream where they migrated to the bone marrow. In the bone marrow the cells appeared to take on their designated role of producing ADA. The second child to receive this treatment was three-day-old Zachary Riggins. Both cases appeared to be successful. Scientist are hopeful that this method will provide a cure for those obtaining prenatal diagnosis. This procedure theoretically should provide long term expression of ADA with no adverse side affects but only time will tell.

ILLUSTRATIONS 6

The cover illustration for SCID is an 11"X14" acrylic painting on gessoed water color paper. It was created like the previous ones with a series of washes built up over a fixed pencil illustration. As I began the research of the disease I searched through books for photos of patients with the disease. The most common one was David the "Bubble boy". David's struggle with the disease had become fairly well known, he had even been mentioned in a song by Paul Simon. The public's knowledge of David and their sympathy for him would make it easier for them to identify with the disease, drawing their interest to the article.

In the layout of this piece I wanted David to be the main focus bringing him close to the picture plane, but also indicate his separation from us (the world). The application of paint in the treatment of David is more opaque and done in a manner that there is no question that he is the focus of the illustration. There is no visible indications of the disease so I focused more on portraying the seclusion and separation of its victims from the outside world. By widening the view and illustrating more then just his face, I was able to set him back a little from us. This also allowed me to give some indication of his hands at the bottom of the piece in thick rubber gloves, the only part of him able to leave the bubble. His head cocked back slightly and turned, with his eyes looking off also helps separate him. It suggests an unawareness of our presence.

Transparent white washes were selectively applied over his face to indicate the plastic bubble. The background was kept subdued using earthy tones with subtle indications of an environment. This was also set back by rubbing a fine grade sand paper over it. A stem cell was added in the top right corner to indicate the focus of the new treatments for the disease. The red blood cells surrounding it act as more of a supportive element. They help to identify the stem cell, and set it apart from the background which is very similar in color. Their replication and placement adds a pleasing visual effect. White hand lettering was used as in the Huntington's piece. It was applied in thin layers allowing hints of the background to show through.

ILLUSTRATION 7

The computer piece was created similar to the others in which a black and white pencil illustration was brought into Photoshop 3.0 and colorized. The colorized file was then brought into Quark 3.32 for layout. The illustration is more informative then the cover illustration. It acts as a visual aid, indicating the new methods of gene therapy being applied to SCID patients as discussed in the article.

I used the same type of background as in the previous piece but modified the color scheme to pick up the blue tones from the cover illustration. The infant is the main focus of this illustration showing the new treatments utilizing umbilical blood. Its large scale and central placement indicate its importance. The treatment is shown in a series of steps directed by arrows. These steps start with the removal of umbilical blood with its stem cells, viral transfer to the stem cell nucleus and eventually the return of the cell to the baby. The arrows used to direct the sequence of the procedure also play a part in keeping the infant central by their circular formation around his head. The vertebra section below his foot indicates the success of the treatment with the stem cell's return to the bone marrow and the production of healthy T-cells. The illustration is tied together by its movement around the page and variations between foreground and

background images. Fading images into the background works well in setting them back, for example the gene transfer procedures above and behind the infant's head offers enough information to make the procedure clear but doesn't compete with him for attention. Other images are raised from the background by laying shadows behind them thus giving a feeling that they are floating.

The page layout follows the same format as the previous ones with the illustration bleeding off the top corner of the page, two columns of 10 point Futura type for the body of the article, a small block of 10 point times italic describing the illustration, and the title running vertically up the left side. The only exception is that this title includes the full name of the disease. Because of this addition I reduced the size of the font used on the full name and change it to Futura to prevent it from appearing busy and hard to read.

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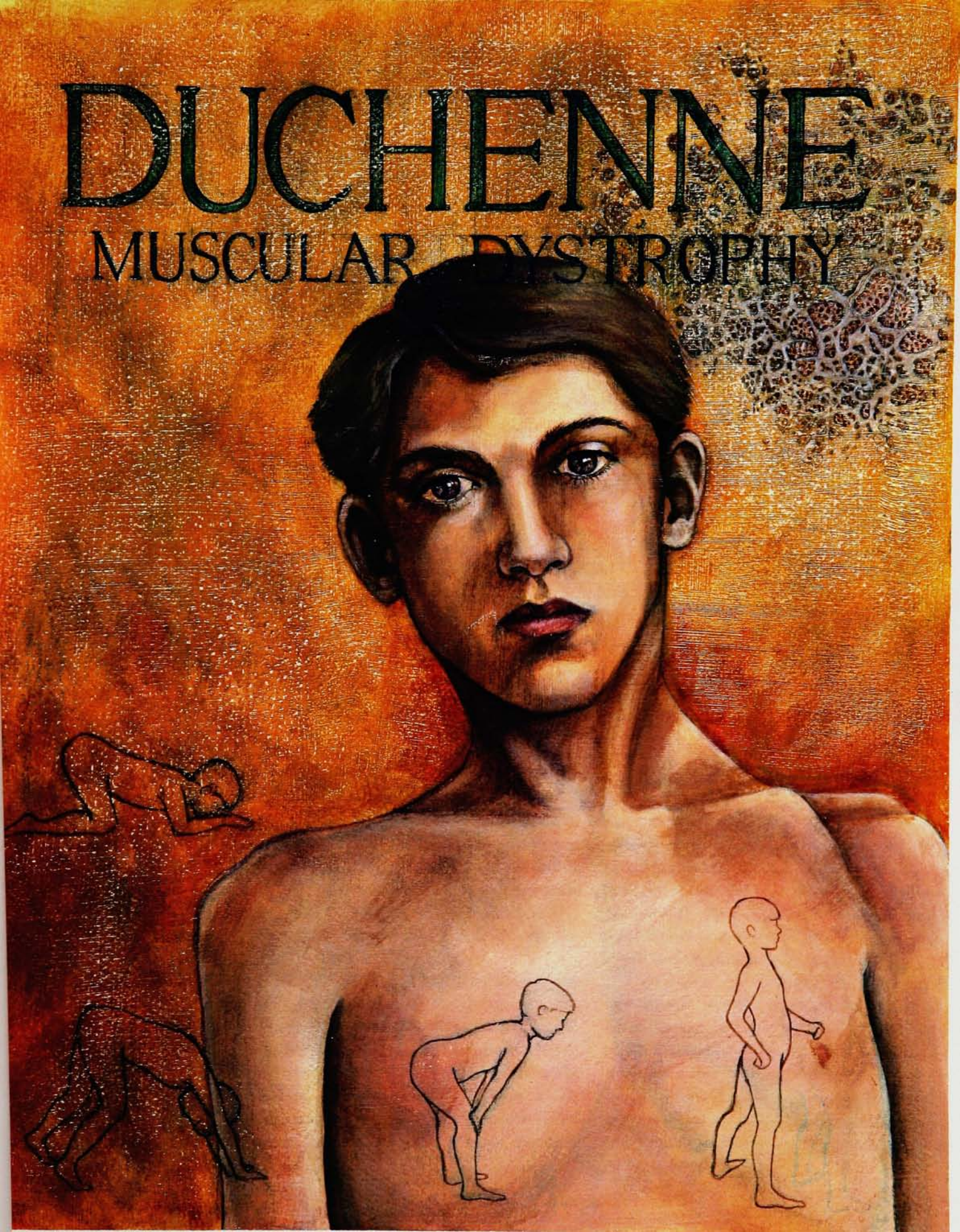
DUCHENNE
MUSCULAR DYSTROPHY

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Duchenne's Muscular Dystrophy

Myoblasts containing normal copies of the dystrophin gene are injected into the patient's leg muscles.
The myoblasts bind to the patient's cells, and differentiate into muscle fibers, capable of producing dystrophin.

Duchenne's muscular Dystrophy (DMD) is the most common inherited skeletal muscular disease, affecting 70 percent of the body's muscle cells. It expresses itself early on, causing a progressive decay of skeletal muscles and a significant drop in muscle replication. The disease usually detectable within the first two years, confines its victims to a wheel chair by age 13, and ends in death by 20.

DMD has been linked to a mutation of the gene responsible for production of the cytoskeletal protein, dystrophin. Dystrophin, present in the muscle sarcolema, functions as an important structural element to the muscle membrane. The absence of this protein causes weakening of molecular connections and eventual rupture of the membrane due to stress in muscle contraction.

Experimental treatment for DMD is based on a belief that the introduction of normal versions of the dystrophin gene could prevent expression of the disease. The regenerative properties of myoblasts, muscle cell precursors, are ideal for the transferring the gene. Theoretically the myoblasts would bind to the patients cells and differentiate into muscle fibers capable of producing dystrophin.

Dr Peter Law brought this concept to human trial. He first injected patients with an immune suppressive drug to prevent cell rejection. Myoblasts containing the dystrophin gene were then injected into each of the 22 major muscles of the leg. The patients exhibited a production of dystrophin, and improved muscle strength. These results provide hope that a cure is near.

Duchenne's Muscular Dystrophy
DMD

Duchenne's Muscular Dystrophy (DMD) is the most common inherited skeletal muscular disease affecting more than 1 in 3500 males. It is an X-linked recessive disease causing deterioration of 70 percent of the body's muscle cells. It is expressed early on, usually detectable by the time the child begins to walk. From this point there is a progressive decay of skeletal muscles. A significant drop in muscle replication is evident by age 2. The disease usually confines its victims to a wheelchair by age 13 and results in death by age 20. Progress in genetic research offers new hope for these patients. The discovery of the gene responsible for causing the disease has made experimentation with gene therapy possible.

In 1987 Koening identified the sequence of the gene affected in DMD patients, this is the largest sequence identified in the human genome to date. The gene is responsible for the coding of a rod-shaped protein, called dystrophin. DMD was the first type of muscular dystrophy whose mutated gene was cloned and the protein it codes for identified. The discovery has led to more concentrated research of the disease, making it the most focused on of muscular diseases. 60 percent of individuals expressing symptoms of DMD exhibit a deletion in the dystrophin gene, and 5 percent show a duplication mutation. Mutation of the gene is usually the result of a stop codon which causes premature termination of the polypeptide and affects the production of dystrophin. The dystrophin protein present in the muscle sarcolema, functions as an important structural element of the muscle membrane. The absence of this protein causes a weakening of molecular connections and eventual rupture of the membrane during the stress of muscle contraction. Cell death is easily detectable by measuring the levels of an enzyme called creatine kinase in the blood. Small amounts of this enzyme, responsible for maintaining muscle energy levels is normally detectable in the blood. It is released in large amounts in DMD patients leaking from the muscle

tissue being broken down.

The severity of this disease, for which there had been no adequate treatment and no hope of stabilization or improvement, has made it a major interest in genetic research. The development of mouse and canine models has provided much insight into the possibilities of gene therapy treatments for the disease. The mice studied have been bred with simulated DMD, showing no expression of dystrophin in skeletal and cardiac muscle. The muscles of these mice began to show signs of necrosis and regeneration leading to centralized nuclei in the majority of muscle cells. The centralized nuclei indicate ongoing muscle degeneration and regeneration. After 20 days atrophy and fibrosis similar to that of DMD patients occurred. The difference in the mice however, was that they demonstrate no weakness. The mouse model offers an accurate simile of the disease's chemical and genetic expression but it differs in its physical expression. Dogs used in the canine models do not contain dystrophin, exhibit muscular weakness, similar to that expressed in humans. Using these two models researchers began experimenting with gene therapy for methods of curing the disease.

Experimental treatment for DMD was based on a belief that the introduction of a normal version of the dystrophin gene could prevent expression of the disease. One important consideration in these experiments was the size of the dystrophin gene. The full length of the gene was too large to be carried in the common adenoviral or retroviral vectors. To get around this problem minigenes were created that were short enough in length to fit in the virus, yet retained the information necessary to produce dystrophin. Based on this model, two human minigenes were created for experimentations in gene transfer. In a series of these experiments copies of the minigene were injected into fertilized mouse ova that had inherited the defective dystrophin gene. As the cells multiplied so did the correct copy of the gene so that it was expressed in every cell of the body. As a result the mice were born with no expression of the disease. This study provided hope that the transfer of correct versions of the dystrophin gene can relieve the expression of the disease. Their theory was supported by the expression of

dystrophin in the sarcolemal membrane, decrease in the number of fibers with centralized nuclei, and restoration of dystrophin associated proteins. Similar techniques were performed in canine experiments in which researchers were able to get a better idea of the experiments effects on the phenotype.

With indications that a transfer of a healthy gene to the cells could cure the disease, methods of transferring the gene to the appropriate cells because the biggest dilemma. Successful gene transfer for human trials requires that the normal versions of the gene be transferred to at least 70 percent of the muscle cells in the body. This statistic rules out direct injection as an effective method because the cells would only be expressed at the site of the injection. Viral vector systems which can transport truncated versions of the gene are more efficient. A retrovirus could only be be used to transfer the gene into myoblasts because they infect dividing cells . The myoblast would then have to be transferred to the muscle. Adenoviruses, on the other hand, do not require cell division and can transfer the truncated gene directly to the muscle.

The current procedure for the therapy includes the use of the myoblasts (muscle precursor cells). The new vector system utilizes the regenerative property of myoblasts to deliver the functioning dystrophin gene to the muscle. Theoretically, the muscle cells produced by the myoblast would fuse to the patients muscle and each other, and the new cells would be equipped to produce the dystrophin needed to strengthen the muscles. Results indicate that the new cells were producing the needed dystrophin but the dystrophin expression was short lived.

Human trials for the treatment of gene therapy on DMD patients is being performed at several centers throughout the world. One of the leaders of this treatment is Dr. Peter Law, director of the Cell Therapy Research Foundation in Memphis. Using a myoblast transfer method, Law performed the procedure on 24 DMD children. The children were first injected with cyclosporine, an immune suppressive drug to reduce rejection of the transfer. Myoblasts containing the normal dystrophin gene were collected from healthy individuals. Five billion of the healthy cells were injected into

each of the 24 major muscles of the leg of each child. The patients treated began to exhibit a production of dystrophin and increased muscle strength. 81 percent of the injected muscles appeared to get stronger, some patients, who were wheelchair bound prior to the expression, were even able to walk . The drawback to this method however, is that repeated injections could have an adverse effect, weakening the already affected muscles.

Therence Partridge of London's Caring Cross Hospital performed a variation of this experiment in which he used myoblasts extracted from DMD patients. The myoblasts were injected with normal copies of the gene. The cells were multiplied in culture, and then reinjected. His results were similar to Law's but, he observed that the dystrophin was only expressed local to the site of injection. In order for complete muscle restoration injections would have to be given all over the body and repeated injections may also be required. Injection into the blood stream would solve the problem of localization of the cells. Experiments of this type however, show that the cells have a difficult time leaving the blood and entering the muscle. Patridge is currently working on engineering cells that would have similar characteristics as the white blood cells in their ability of leaving the blood stream and attaching to muscle in immune system responses.

There still is no effective treatment for DMD but greater understanding of the disease and experimentation and trials with gene therapy offer a new hope for these children.

ILLUSTRATIONS 8

The cover illustration for Duchenne's Muscular Dystrophy is an 11"X14" acrylic painting on gessoed paper. It was created in the same style as the previous cover illustrations, by building up washes over a fixed pencil drawing. Keeping with a similar color scheme as the previous pieces I chose earthy tones of gold and brown for the

background and repeated them in the figure as well.

I began collecting information on the disease and looking for photo sources, most of which were young boys due to the early expression of this X-linked disease. One of the main sites of expression of the disease is the leg muscles so many of the sources showed full body shots of the boys. I wanted to stick with the idea of keeping the illustration more personal. Coming in closer on the patient makes it a little more intimate and easier for the viewer to relate to the patient, thus creating an interest. Showing the full figure would set the image back too far, making it seem more like a case study. I did decide to widen the the view a little to include the shoulders and chest. The strain in the neck and shoulders gives some indication of the loss of muscle control. Although I widened the view one factor that helps to keep the image up front is the boys eyes, they stare right out at you and helping to draw the viewer in.

I used sketchy schematic figures along the bottom of the illustration to provide the information lacking in th portrait. These types of figures appeared in most of the material I researched. The figures show the steps the patients go threw in standing up. This four or more step process is common among all DMD patients because they don't have the leg strength to pull themselves up the way most people do. No more information then an outline is necessary to convey the information and the outline works well with the piece because it allows the background to show through so as not to compete with the portrait. The image in the upper right corner is a histology plate of muscle. I added this more as a design element then a source of information. It fills the area nicely forming an interesting pattern. I chose a palette of browns for the muscle so that it would not become to heavy in the corner, this also made it easy to fade into the background. Because of the light gold background I choose a darker color for the type. To break the monotony of browns I used a rich green. The green was applied in a similar manner as in the previous illustrations with thin washes so that some of the information behind was allowed to show through. When the painting was complete I went over it with a fine grain sand paper to unite it. The boy was left untouched

keeping him the focal point.

ILLUSTRATION 9

The computer piece was created the same way as the previous ones by scanning a black and white pencil illustration into Photoshop 3.0 and colorizing it. The colorized file was then brought into Quark 3.32 to create the layout. The images were placed on different layers to give more of a sense of depth and make them easier to work with.

I used the same type of background as in my previous computer pieces but changed the colors to pick up the golden browns from the painting. The illustration is meant to show the procedure of myoblast transplants which focuses on the leg muscles of DMD patients. I made the leg a major element of the illustration by increasing its size and bringing it to the foreground. The other essential element in this procedure is the myoblast containing the healthy dystrophin gene being transferred. The three myoblasts in the upper right corner were added as an aesthetic decision. One myoblast due to its long thin shape seemed to get lost in the corner, and enlarging it competed too much with the leg. Angling one myoblast helps break the verticals and draw the eye around. The layout helps to direct the viewer through the sequence of the steps in the procedure, from the myoblast to the hypodermic needle to the leg. The muscle bundle coming out of the leg helps to orient the viewer between the gross view and the microscopic, where the process is being carried out. I used extreme foreshortening because of the small size of the myofiber. It also helps in drawing the eye to the lower right section of the illustration. The image of the fibers in the lower right show the results of the procedure with myoblasts containing the healthy gene migrating and forming new muscle fibers. I dropped the image to the background to give some depth variation. The fiber crossing over it allows enough information to be seen for it to be identifiable without over emphasizing it. The cross section of muscle

fibers above it that fade into the background were an aesthetic decision to help unite the information.

The layout for the piece is similar to the others, the illustration bleeds off the top right corner, two columns of 10 point futura type lines up under the illustration, and a small block of 10 point Times italic on the left side describes the illustration. The title of the article runs vertical up the side, using a sanserif and smaller font size for the full name of the disease to make it more readable.

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